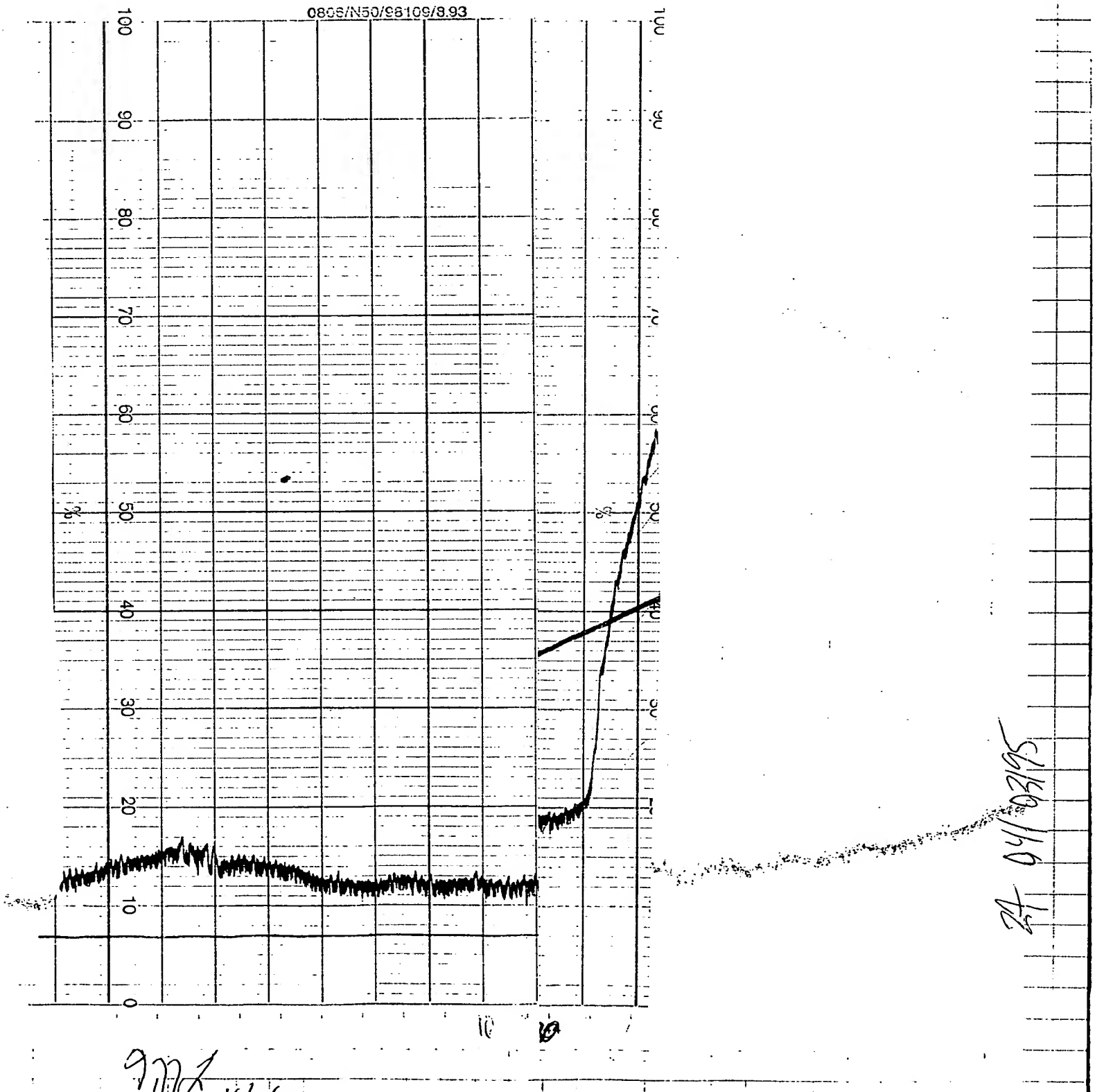
Heparin > Q 650 · True - Purification

Project No.
Book No.

Page No.



MK 4/5/95

To Page No.

Witnessed & Understood by me,	Date	Invented by E. Flynn	Date
Mary Longo	4/5/95	Recorded by	04-103/95

Project No._____
Book No._____ TITLE X-column activity assay -

From Page No.___

Y-column - DEAE cond.

fractions - 20 - 40 + 44

SAM	Fraction	CPM1	
1	20	688.00	
2	21	5432.00	
3	22	40238.00	
4	23	80430.00	Pool 1
5	24	65778.00	
6	25	68530.00	Pool 2
7	26	66598.00	
8	27	61710.00	
9	28	49366.00	
10	29	26334.00	
11	30	18684.00	
12	31	11410.00	
13	32	9328.00	
14	33	6658.00	
15	34	7150.00	
16	35	6624.00	
17	36	6038.00	
18	37	3336.00	
19	38	3926.00	
20	39	2958.00	
21	40	5312.00	
22		161992.00	
23		304.00	
24		93388.00	
25		91384.00	
26		92410.00	

10/13/94

MJ
4/5/95

Premix - (TAQ) 11 µl dNTP / 500 µl @ premix

24 µl aliquot / rxn - .5 µl of fraction (was added - incubated @ 72°C in a water / heat block for 8 minutes - quenched with 10 µl of .5 M ½ EDTA -

20 µl spotted on 6 P/C filter
Washed 1 x 10% TCA 1% DPi
3 x 1% TCA
2 x EtOH
dried under a heat lamp + counted

Storage Buffer - (TAQ)
Bicin
20mM pH 8.0
1 mM EDTA
1 mM DTT
50% glycerol

Dialyze O/N against 2 Liters - @ 4°C

T Page

Witnessed & Understood by me, Mary Longo
Dat 4/5/95
Invented by Elizabeth Hogan
Recorded by
Dat 04/05/95

X-column Bradfuds - 12% PAGE

ag N .

-8-
Conc (mg/ml)

3ug

1	Load	0.541194	9.25	5.3
2		0.591386		
3	20	0.000000		
4	21	4.364472e-3	20	12.5
5	22	0.240046	10.2	6.1
6	23	0.491003	11	6.8
7	24	0.442994	27	16.6
8	25	0.185490	31	16.8
9	26	0.165850	41	25
0	27	0.121478	55	30
1	28	0.098201	55	32.3
2	29	0.093109	71	41
3	30	0.072741	50	28
4	31	0.105838	31	18.4
5	32	0.163668	33	18.8
6	33	0.159303	33	19.4
7	34	0.154939	71	40
8	35	0.075651	55	31
9	M	0.096746	62	37.5
0	37	0.086562		

4/5/95

4/5/95

Loaded on 12% PAGE

2 3 4 5 6 7 8 9 10 1 2 3 4 5 6 7 8 9
LM 22 23 24 25 26 27 28 29 30 31 32 33 34 35 M

4/5/95

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sed & Understood by me,	Date	Invented by	Date
Mary Longo	4/5/95	R c rd d by	04/03/95

Project No._____
118 Book No._____ TITLE Units - on Loads + Pools -

From Page No.__

Purpose: Whd to determin total units on Heparin + Q650 + the total units pooled -
+ determin units / gram from crack sample

1. crude - 1/2000
2. after heat shock 1/2000
3. Load PET
4. Load Hep 1/1000
5. Pool Hep 1/1000
6. Load Q650 1/1000
7. Pool ① Q650 1/500
8. Pool ② Q650 1/500

(7x3) = 21 samples -

Taq Dilution Buffer
25mM tris pH8.0
50mM KCl
100µg/mL gelatin
.1mM EDTA
.5% NP-40
.5% Tween 20
1mM Bme

SAM	CPM1		
1	1958.00	Load Hep	118
2 1/5000	2486.00		42
3	3196.00		48
4	2746.00	Pool Hep	49
5 1/1000	3998.00		7234
6	5108.00		23
7	3000.00	Pool ①	72
8 1/500	4990.00		60
9	5510.00	Pool ②	33
10	4888.00		59
11 1/1000	7964.00		48
12	8240.00		
13	7990.00		
14	10032.00		
15	8612.00		
16	428.00		
17	78186.00		
18	78040.00		
19	79558.00		
20	22.00		
21	26.00		

78594 - x
SA = 49.7

Not too good need to wash

4/5/95

To Page N

Witnessed & Understood by me,	Dat	Invent d by	Date
Mary Longo	4/5/95	Rec rded by	04/04/95